UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: **July 16, 2009**

SUBJECT: **Mancozeb and Ethylene Thiourea.** Livestock Analytical Methods Required in the Generic Data Call-In from the Mancozeb Reregistration Eligibility Decision.

PC Code: 014504; 600016
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: NA
MRID No.: 47358701 through 47358703; 47375201

DP Barcode: D352531
Registration No.: NA
Regulatory Action: RED Follow-Up
Case No.: 0643
CAS No.: 8018-01-7; 96-45-7
40 CFR: 180.176

FROM: Christine L. Olinger, Chemist
Risk Assessment Branch 7
Health Effects Division (7509P)
Office of Pesticide Programs

THRU: Michael S. Metzger, Chief,
Risk Assessment Branch 7
Health Effects Division (7509P)
Office of Pesticide Programs

TO: Christina Scheltema, CRM
Reregistration Branch 3
Special Review and Reregistration Division (7508P)
Office of Pesticide Programs

In response to Generic Data Call-In (GDCI) associated with the Mancozeb Reregistration Eligibility Decision (RED), the Mancozeb Task Force has submitted Independent Laboratory Validations (ILV) for four residue analytical methods used for the determination of mancozeb and the metabolite ethylene thiourea (ETU) residues in meat and milk. The Special Review and Reregistration Division has requested HED review of these studies to determine if they satisfy the data requirement for Guideline No. 860.1340.

The attached Data Evaluation Records (DERs) were originally prepared under contract by Dynamac Corporation (2275 Research Boulevard, Suite 300; Rockville, MD 20850). The DER has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

JUL 20 REC'D, 2009

The Independent Lab Validations indicate that the submitted methods are suitable for the determination of mancozeb and ETU in meat and milk. However, the independent labs recommended minor modifications to the method to allow for different quantitation methods or equipment. The methods should be revised as specified in the Independent Lab Validation and submitted to the Agency.

cc: Lisa Jones, Registration Division

Mancozeb/PC Code 014504/The Mancozeb Task Force
DACO 7.2.1, 7.2.2, and 7.2.3/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method – Meat and Milk (EDBC Methods)

Primary Evaluator Dynamac Corporation Date: 12/29/08

Approved by Date: 7/16/09
Christine Olinger, Chemist, RAB 7

This DER was originally prepared under contract by Dynamac Corporation (2275 Research Boulevard, Suite 300; Rockville, MD 20850. The DER has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORTS:

47358701 Clayton, B. (2000) Independent Laboratory Validation (ILV) of Analytical Method No. ETU-89 AM-003, "Determination of Ethylene BIS Dithiocarbamates (EBDC's) in Milk". Project Number: 00/0025, TR34/00/100. Unpublished study prepared by En-Cas Analytical Laboratories. 65 p.

47358702 Clayton, B. (2000) Independent Laboratory Validation (ILV) of Analytical Method No. ETU-89 AM-002, "Determination of Ethylene BIS Dithiocarbamates (EBDC's) in Meat". Project Number: 00/0023, TR34/00/99, 38165. Unpublished study prepared by En-Cas Analytical Laboratories. 65 p.

EXECUTIVE SUMMARY:

The Residue Chemistry Chapter for the Mancozeb RED (DP# 305815, 6/14/05, C. Olinger), requires submission of enforcement analytical methods for the determination of mancozeb residues in ruminant commodities.

In response, the Mancozeb Task Force, with members consisting of Dow AgroSciences LLC, E.I. du Pont de Nemours and Company, and United Phosphorus, has submitted method descriptions and independent laboratory validation (ILV) data for two gas chromatography methods with flame photometric detection (GC/FPD methods) for the determination of residues of EBDCs, including mancozeb, maneb, and metiram in meat (Method ETU-89AM-002; MRID 47358702) and milk (Method ETU-89AM-003; MRID 47358701). Using these methods, residues of the EBDCs are determined as CS_2. Both ILVs were conducted by EN-CAS Analytical Laboratories (Winston-Salem, NC).

The methods are identical except that meat is prepared for extraction by grinding in the presence of dry ice, and milk is prepared for extraction by thawing to a slushy consistency. Samples are extracted with 10% EDTA, 8 N HCl , and 3% stannous chloride solution. The mixture is reacted for 2 hours in a boiling water bath and then maintained at 100 °C for analysis. An aliquot of the headspace is removed, and residues of mancozeb (EBDC) are determined as CS_2 by GC/FPD.

No method validation data or radiovalidation data were included in the submissions, and no confirmatory procedures were reported.

Acceptable ILV results were obtained for both methods on the first trials using samples of beef muscle and milk, both fortified at 0.01 ppm (limit of quantitation; LOQ) and 0.10 ppm (10x LOQ). The ILV laboratory recommended several changes to the methods.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method test data reflecting ILV of methods ETU-89AM-002 and ETU-89AM-003 are classified as scientifically acceptable. The changes recommended by the ILV laboratory should be incorporated into the methods. For the methods to be acceptable as enforcement methods, additional data may be required pertaining to method validation and/or radiovalidation. In accordance with established CS_2-generating enforcement methods for EBDCs in plant commodities, a confirmatory method is not needed.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP# 352531.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Mancozeb is a List A reregistration chemical; the Reregistration Eligibility Decision was issued 9/2005. Mancozeb, a coordination product of zinc ion and maneb (manganese ethylenebisdithiocarbamate), is a member of the ethylene bisdithiocarbamate (EBDC) group of fungicides and is currently registered for use on a variety of fruit, vegetable, nut, and field crops. The Agency has determined that mancozeb only is to be included in the tolerance expression for mancozeb.

The chemical structure and nomenclature of mancozeb and its metabolite ETU are presented in Table A.1. The physicochemical properties of the technical grade of mancozeb are presented in Table A.2.

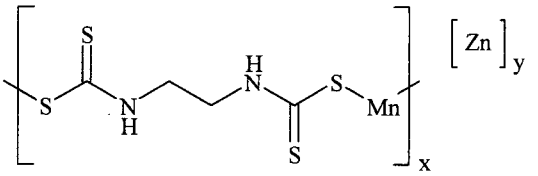
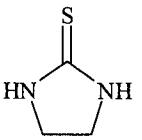
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Mancozeb
Company experimental name	Not applicable
IUPAC name	manganese ethylenebis(dithiocarbamate)(polymeric) complex with zinc salt
CAS name	[[1,2-ethanediylbis[carbamodithioato]](2-)]manganese mixture with [[1,2-ethanediylbis[carbamodithioato]](2-)]zinc
CAS registry number	8018-01-7
Chemical structure of ETU metabolite	 ethylenethiourea

TABLE A.2. Physicochemical Properties of Technical Grade of Mancozeb		
Parameter	Value	Reference
Melting point/range	Decomposes at 150 °C	RCB No. 4368, 11/9/88, G. Makhijani
pH	6.5 (25% slurry in water)	
Density	0.41 g/cc (packed)	
Water solubility	1.1 µg/mL at room temperature	
Solvent solubility	Insoluble in most organic solvents	DEB No. 4689, 12/16/88, G. Makhijani
Vapor pressure	Not applicable; negligible	RCB No. 4368, 11/9/88, G. Makhijani
Dissociation constant, pK_a	Not applicable; does not dissociate	DEB No. 4750, 1/24/89, G. Makhijani
Octanol/water partition coefficient, $Log(K_{OW})$	3.24 at room temperature	RCB No. 4368, 11/9/88, G. Makhijani
UV/visible absorption spectrum	Not available	

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

Not applicable to this submission.

B.2. Enforcement Method

The Mancozeb Task Force, with members consisting of Dow AgroSciences LLC, E.I. du Pont de Nemours and Company, and United Phosphorus, has submitted method descriptions and ILV data for two GC/FPD methods for the determination of residues of EBDCs, including mancozeb, maneb, and metiram in meat (Method ETU-89AM-002; MRID 47358702) and milk (Method ETU-89AM-003; MRID 47358701).

B.2.1. Principle of the Method:

The parameters of methods ETU-89AM-002 and ETU-89AM-003 are described in Table B.2.1. The methods are identical except that meat is prepared for extraction by grinding in the presence of dry ice, and milk is prepared for extraction by thawing to a slushy consistency. Samples are to be kept frozen at all times until addition of extraction/reaction reagents.

Samples are extracted with 10% EDTA, 8 N HCl , and 3% stannous chloride solution. The mixture is reacted for 2 hours in a boiling water bath and then maintained at 100 °C for analysis. An aliquot of the headspace is removed, and residues of mancozeb (EBDC) are determined as CS_2 by GC/FPD.

TABLE B.2.1. Summary Parameters for the Analytical Enforcement Methods Used for the Quantitation of Mancozeb (EBDC) Residues in Milk and Beef Muscle.

Method ID	ETU-89AM-002 (meat; MRID 47358702)	ETU-89AM-003 (milk; MRID 47358701)
Analyte	Mancozeb and other EBDCs including maneb and metiram; determined as CS_2	
Extraction solvent/technique	Meat is homogenized by grinding in the presence of dry ice.	Milk is quick-thawed to a slush using a cold water bath.
	Residues are extracted with 10% EDTA, 8 N HCl, and 3% stannous chloride solution. The mixture is reacted for 2 hours in a boiling water bath and then maintained at 100 °C in the water bath during GC/FPD determination of any EBDC (maneb, mancozeb, or metiram) residues as CS_2. Samples must be reacted immediately following addition of reaction reagents; once reacted, samples may be stored overnight at room temperature and reheated to 100 °C the following day for determination.	
Cleanup strategies	None.	
Instrument/Detector	Gas chromatography with flame photometric detector (GC/FPD) in the sulfur mode.	
Standardization method	External standardization using a standard curve prepared by prepared by plotting amount injected versus peak height on log/log graph paper.	
Stability of std solutions	Standards are prepared as needed; the least stable EBDC standard is stable in water for 30 minutes, and all manipulations with suspension standards must be completed within this time frame.	
Retention times	Mancozeb (as CS_2): 6 minutes	

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

Not applicable to this submission.

C.2. Enforcement Method

No method validation data or radiovalidation data were included in the submissions, and no confirmatory procedures were reported. The method characteristics for methods ETU-89AM-003 and ETU-89-AM-002 are presented in Table C.2.2.

TABLE C.2.1. Recovery Results from Method Validation of Milk and Beef Muscle using the Enforcement Analytical Method.			
Matrix	Spiking Level (ppm)	Recoveries Obtained	Mean Recovery ± Std. Dev. [CV] (%)
Not applicable; no method validation data were submitted.			

TABLE C.2.2. Characteristics for the Enforcement Analytical Methods Used for the Quantitation of Mancozeb (EBDC) Residues in Milk and Beef Muscle.		
Method ID	ETU-89AM-002 (meat; MRID 47358702)	ETU-89AM-003 (milk; MRID 47358701)
Analyte	Mancozeb and other EBDCs including maneb and metiram; determined as CS_2	
Equipment ID	Microtek MT220 or equivalent gas chromatograph with flame photometric detector in the sulfur mode and glass column packed with PT 28% Alltech 223 + 4% KOH on 80/100 Gas Chrom R or 28% Pennwalt 223 + 4% KOH on 80/100 Gas Chrom R.	
Limit of quantitation (LOQ)	0.01 ppm	
Limit of detection (LOD)	0.002 ppm	
Accuracy/Precision	No method validation data were provided. ILV recoveries indicated acceptable accuracy/precision, ranging 72.9-108% (average = 87.7%; CV = 15.9%); refer to Table C.3.1.	No method validation data were provided. ILV recoveries indicated acceptable accuracy/precision, ranging 68.5-91.4% overall (average= 76.7%; CV = 9.9%); refer to Table C.3.1.
Reliability of the Method [ILV]	ILV Study No. 00-0023 was conducted to verify the reliability of the method for the determination of mancozeb residues in meat. The values obtained are indicative that the method is reliable. Refer to Table C.3.1.	ILV Study No. 00-0025 was conducted to verify the reliability of the method for the determination of mancozeb residues in milk. The values obtained are indicative that the method is reliable. Refer to Table C.3.1.
Linearity	The method/detector response was linear (coefficient of determination, r^2= 0.999) within the range of 0.2-1.0 ng.	The method/detector response was linear (coefficient of determination, r^2= 0.999) within the range of 0.4-1.4 ng.
Specificity	The control chromatograms generally have no peaks above the chromatographic background, and the spiked sample chromatograms contain no interfering peaks. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.	

C.3. Independent Laboratory Validation

ILV studies for methods ETU-89AM-002 and ETU-89AM-003 were conducted by EN-CAS Analytical Laboratories (Winston-Salem, NC). Samples of untreated beef muscle and milk (both obtained locally) were fortified with mancozeb at 0.01 and 0.10 ppm (LOQ and 10x LOQ). Five samples at each fortification level were analyzed by the method procedures described in Table B.2.1.

The laboratory made the following minor modifications to the methods for the ILV trial: (1) use of a different GC system; (2) increasing the lowest calibration standard concentration; (3) use of check standards not specified in the method to monitor instrument performance; and (4) use of a reagent blank in each sample set to monitor any effect the reagents may have had on results.

The ILV was successful on the first trial for each matrix; recoveries are reported in Table C.3.1.

The ILV laboratory identified one critical step: the EBDC standard forms a suspension in water and must be stirred while aliquots are taken for dilutions or fortifications; all manipulations with the suspension standards must be completed within 30 minutes due to stability concerns.

The ILV laboratory recommended the following changes to the method: (1) replace specific values for the lowest standard on the 4-point standard curve with a statement that the lowest standard should be 50% of the LOQ equivalent; (2) replace the recommended minimum peak height of 8 mm with a recommendation that the lowest standard peak should be well resolved and distinct from the baseline; (3) add a statement for the standard curve that a power curve ($y = ax^b$) may be used for calculation of data; (4) add the use of check standards injected periodically to monitor instrument performance; and (5) indicate that a reagent blank may be included to monitor any reagent effects.

The ILV laboratory reported that a set of seven samples can be prepared by one analyst for GC/FPD analysis in one half-day (~4 hours) and a GC/FPD run containing six standards and seven samples can be completed in 3 hours, requiring manual injections.

The ILV laboratory reported that permission was obtained from the Sponsor Study Monitor for the method modifications made prior to the beginning of analysis, and that the Study Monitor was notified of the results of each sample set as they were completed. Documentation of communications was not included in the submission.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Methods for the Determination of Mancozeb (EBDC) in Milk and Beef Muscle.

Matrix	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± Std. Dev. [CV] (%)
ETU-89AM-002: Beef muscle	0.01	87.5, 93.0, 103, 106, 108	99.5 ± 8.8 [8.9]
	0.10	72.9, 74.3, 75.4, 77.9, 78.5	75.8 ± 2.4 [3.1]
ETU-89AM-003: Milk	0.01	74.0, 74.5, 79.4, 85.7, 91.4	81.0 ± 7.5 [9.3]
	0.10	68.5, 68.9, 71.6, 71.8, 81.1	72.4 ± 5.1 [7.0]

D. CONCLUSION

Adequate ILV data have been submitted for GC/FPD methods ETU-89AM-002 and ETU-89AM-003, reflecting analysis of beef muscle and whole milk samples, respectively, both fortified at 0.01 ppm (LOQ) and 0.10 ppm (10x LOQ). samples. The ILV laboratory recommended several changes to the methods. No method validation or radiovalidation data were included in the submissions, and no confirmatory procedures were reported.

E. REFERENCES

DEB No.: 4368
Subject: Mancozeb – Unregistered Technical – Rohm & Haas Company – Product Chemistry Data Dated August 30, 1988.
From: G. Makhijani
To: L. Rossi and R. Engler
Date: 11/9/88
MRIDs: None

DEB No.: 4689
Subject: Mancozeb – Unregistered Technical – Rohm & Haas Company – Product Chemistry Data Dated November 18, 1988.
From: G. Makhijani
To: L. Rossi and R. Engler
Date: 12/16/88
MRIDs: 40898301 and 40898302

DEB No.: 4750
Subject: Mancozeb – Unregistered Technical and Manufacturing Use Products – E.I. DuPont de Nemours and Company, Inc. – Product Chemistry Data Dated December 2 1988.
From: G. Makhijani
To: L. Rossi and R. Engler
Date: 12/16/88
MRIDs: None

DP#: 305815
Subject: Mancozeb: Residue Chemistry Chapter of the Reregistration Eligibility Decision; Chemical No. 014504
From: C. Olinger
To: K. Farwell and T. Spears
Dated: 6/14/05
MRIDs: None

F. DOCUMENT TRACKING

RDI: COlinger (7/6/09)
Petition Number: Not Applicable
DP#: 352531
PC Code: 014504

Template Version June 2005

Mancozeb/PC Code 014504/EBDC/ETU Task Force
DACO 7.2.1, 7.2.2, and 7.2.3/OPPTS 860.1340/OECD IIA 4.2.5, 4.2.6 and 4.3
Residue Analytical Method – Meat and Milk (ETU Methods)

Primary Evaluator Dynamac Corporation Date: 12/29/08

Approved by Date: 7/16/09
Christine Olinger, Chemist, RAB 7

This DER was originally prepared under contract by Dynamac Corporation (2275 Research Boulevard, Suite 300; Rockville, MD 20850). The DER has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORTS:

47358703 Clayton, B. (2001) Independent Laboratory Validation (ILV) of Analytical Method # Meth-128, Original, "Determination of Ethylene Thiourea in Milk". Project Number: 00/0026, TR34/00/104, 397. Unpublished study prepared by En-Cas Analytical Laboratories. 92 p.

47375201 Clayton, B. (2001) Independent Laboratory Validation (ILV) Analytical Method # Meth-127, Original, "Determination of Ethylene Thiourea in Meat". Project Number: 00/0024, TR34/00103. Unpublished study prepared by En-Cas Analytical Laboratories. 107 p.

EXECUTIVE SUMMARY:

The Mancozeb Task Force, with members consisting of Dow AgroSciences LLC, E.I. du Pont de Nemours and Company, and United Phosphorus, has submitted method descriptions and independent laboratory validation (ILV) data for two high performance liquid chromatography methods with electrochemical detection (HPLC/ELCD methods) for the determination of residues of ETU in meat (Meth-127; MRID 47375201) and milk (Meth-128; MRID 47358703). Both ILVs were conducted by EN-CAS Analytical Laboratories (Winston-Salem, NC).

The methods are essentially the same except that different extraction solvents are used (ethanol/water for Meth-127 and methanol for Meth-128) and Meth-127 includes an additional clean-up procedure for meat samples containing >15% fat. Frozen samples are combined with 10% Na_2SO_3 and water (meat) or 10% Na_2SO_3 (milk) and adjusted to pH 11-12 with NH_4OH. Sodium chloride, Celite, and ethanol (meat) or methanol (milk) are added, and the mixtures are blended and filtered (beef) or stirred and centrifuged (milk). The resulting filtrate/supernatant is adjusted to pH 7-9 with NH_4OH if necessary, ethylene glycol keeper solution is added, and the sample is concentrated by evaporation. For meat samples containing >15% fat, the extract is partitioned twice between hexane and water to remove the fat; the phases are separated by centrifugation, the hexane phase is discarded, and the aqueous phase is concentrated. For all meat samples, water is added to the aqueous phase/extract, which is then applied to an alumina column; residues are eluted with 2% methanol in dichloromethane (DCM). Ethylene glycol keeper solution is added to the eluate, and the mixture is concentrated and adjusted to volume with water for HPLC analysis. Both methods specify confirmatory analysis on an alternate column with significantly different polarity.

No method validation data or radiovalidation data were included in the submissions.

Acceptable ILV results were obtained for Meth-127 and Meth-128 using samples of ground beef and milk, respectively, both fortified at 0.001 ppm (limit of quantitation; LOQ) and 0.01 ppm (10x LOQ). The ILV laboratory recommended several changes to the methods.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the analytical method test data reflecting ILV of Meth-127 and Meth-128 are classified as scientifically acceptable. The changes recommended by the ILV laboratory should be incorporated into the methods.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP# 352531.

COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

A. BACKGROUND INFORMATION

Mancozeb is a List A reregistration chemical; the Reregistration Eligibility Decision was issued 9/2005. Mancozeb, a coordination product of zinc ion and maneb (manganese ethylenebisdithiocarbamate), is a member of the ethylene bisthiocarbamate (EBDC) group of fungicides and is currently registered for use on a variety of fruit, vegetable, nut, and field crops. The Agency has determined that mancozeb only is to be included in the tolerance expression for mancozeb, but that both mancozeb and its degradate ETU are to be considered for risk assessment.

The chemical structure and nomenclature of mancozeb and its metabolite ETU are presented in Table A.1. The physicochemical properties of the technical grade of mancozeb are presented in Table A.2.

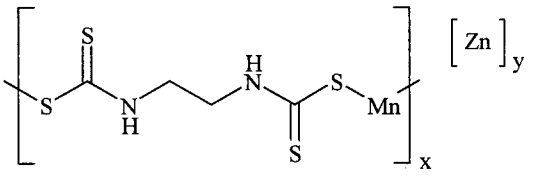
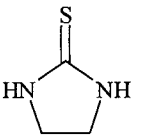
TABLE A.1. Test Compound Nomenclature.	
Chemical structure	
Common name	Mancozeb
Company experimental name	Not applicable
IUPAC name	manganese ethylenebis(dithiocarbamate)(polymeric) complex with zinc salt
CAS name	[[1,2-ethanediylbis[carbamodithioato]](2-)]manganese mixture with [[1,2-ethanediylbis[carbamodithioato]](2-)]zinc
CAS registry number	8018-01-7
Chemical structure of ETU metabolite	 ethylenethiourea
CAS registry number	96-45-7

TABLE A.2. Physicochemical Properties of Technical Grade of Mancozeb		
Parameter	Value	Reference
Melting point/range	Decomposes at 150 °C	RCB No. 4368, 11/9/88, G. Makhijani
pH	6.5 (25% slurry in water)	
Density	0.41 g/cc (packed)	
Water solubility	1.1 µg/mL at room temperature	
Solvent solubility	Insoluble in most organic solvents	DEB No. 4689, 12/16/88, G. Makhijani
Vapor pressure	Not applicable; negligible	RCB No. 4368, 11/9/88, G. Makhijani
Dissociation constant, pK_a	Not applicable; does not dissociate	DEB No. 4750, 1/24/89, G. Makhijani
Octanol/water partition coefficient, $Log(K_{OW})$	3.24 at room temperature	RCB No. 4368, 11/9/88, G. Makhijani
UV/visible absorption spectrum	Not available	

B. MATERIALS AND METHODS

B.1. Data-Gathering Method

B.1.1. Principle of the Method:

Not applicable to this submission.

B.2. Enforcement Method

The Mancozeb Task Force, with members consisting of Dow AgroSciences LLC, E.I. du Pont de Nemours and Company, and United Phosphorus, has submitted method descriptions and ILV data for two HPLC/ELCD methods for the determination of residues of ETU in meat (Meth-127; MRID 47375201) and milk (Meth-128; MRID 47358703)

B.2.1. Principle of the Method:

The parameters of methods Meth-127 and Meth-128 are described in Table B.2.1. The methods are essentially the same except that different extraction solvents are used (ethanol/water for Meth-127 and methanol for Meth-128) and Meth-127 includes an additional clean-up procedure for meat samples containing >15% fat.

Frozen samples are combined with 10% Na_2SO_3 and water (meat) or 10% Na_2SO_3 (milk) and adjusted to pH 11-12 with NH_4OH. Sodium chloride, Celite, and ethanol (meat) or methanol (milk) are added, and the mixtures are blended and filtered (beef) or stirred and centrifuged (milk). The resulting filtrate/supernatant is adjusted to pH 7-9 with NH_4OH if necessary, ethylene glycol keeper solution is added, and the sample is concentrated by evaporation. For meat samples containing >15% fat, the extract is partitioned twice between hexane and water to remove the fat; the phases are separated by centrifugation, the hexane phase is discarded, and the aqueous phase is concentrated. For all meat samples, water is added to the aqueous phase/extract, which is then applied to an alumina column; residues are eluted with 2% methanol in DCM. Ethylene glycol keeper solution is added to the eluate, and the mixture is concentrated and adjusted to volume with water for HPLC analysis. Both methods specify confirmatory analysis on an alternate column with significantly different polarity.

We note that the copy of method Meth-128 included in MRID 47358703 is missing page 3.

TABLE B.2.1. Summary Parameters for the Analytical Enforcement Methods Used for the Quantitation of ETU Residues in Milk and Beef Muscle.

Method ID	Meth-127 (meat; MRID 47375201)	Meth-128 (milk; MRID 47358703)
Analyte	Ethylene thiourea (ETU)	
Extraction solvent/technique	Frozen ground samples are combined with 10% Na_2SO_3 and water and adjusted to pH 11-12 with NH_4OH. NaCl, Celite, and ethanol are added, and the mixture is blended and filtered through Celite. The filtrate is brought to volume with water.	Frozen samples are extracted with 10% Na_2SO_3, stirred, and adjusted to pH 11-12 with NH_4OH. NaCl, Celite, and methanol are added, and the mixture is shaken and brought to volume with methanol, then centrifuged
	The filtrate or supernatant is adjusted to pH 7-9 with NH_4OH if necessary, ethylene glycol keeper solution is added, and the sample is concentrated by evaporation.	

TABLE B.2.1. Summary Parameters for the Analytical Enforcement Methods Used for the Quantitation of ETU Residues in Milk and Beef Muscle.		
Cleanup strategies	If the sample contains >15% fat, the extract is partitioned 2x between hexane and water, then centrifuged. The hexane phase is discarded and the aqueous phase is concentrated.	
	Water is added to the aqueous phase/extract, which is then applied to an Alumina column; residues are eluted with 2% methanol in DCM. Ethylene glycol keeper solution is added to the eluate, and the mixture is concentrated and adjusted to volume with water for HPLC analysis.	
Instrument/Detector	HPLC using a reverse-phase C8 column, an electrochemical detector (ELCD), and an isocratic mobile phase of 0.5% methanol in 0.0218 M H_3PO_4.	
	For confirmatory analysis, a cyano column is used	For confirmatory analysis, a reverse-phase amide column is used.
Standardization method	External standardization using a four-point power ($y = ax^b$) calibration curve.	
Stability of std solutions	The ETU standard solutions appeared to be stable in water for at least one month (MRID 47358703) or 1.5 months (MRID 47375201) when stored refrigerated in the dark.	
Retention times	~5 minutes	~4.5 minutes

C. RESULTS AND DISCUSSION

C.1. Data-Gathering Method

Not applicable to this submission.

C.2. Enforcement Method

The method characteristics for methods Meth-128 and Meth-127 are presented in Table C.2.1.

TABLE C.2.1. Characteristics for the Enforcement Analytical Methods Used for the Quantitation of ETU Residues in Milk and Beef Muscle.		
Method	Meth-127 (meat; MRID 47375201)	Meth-128 (milk; MRID 47358703)
Analyte	ETU	
Equipment ID	Thermo Separation Products SP8800 HPLC equipped with a Bio-analytical Systems LC-4B amperometric ELCD and a Zorbax RX-C8 column (25 cm x 4.6 mm i.d., 5 µ particle size).	
	For confirmatory analysis, a Zorbax SB-CN column (25 cm x 4.6 mm, 5 µ particle size) is used.	For confirmatory analysis, a Zorbax Bonus-RP column (25 cm x 4.6 mm, 5 µ particle size) is used.
Limit of quantitation (LOQ)	0.001 ppm	
Limit of detection (LOD)	Not reported	
Accuracy/Precision	ILV recoveries indicated acceptable accuracy/precision, ranging 69.2-88.3% overall (average = 74.9%; CV = 8.3%) Refer to Table C.3.1.	ILV recoveries indicated acceptable accuracy/precision, ranging 73.1-96.1% overall (average = 84.2%; CV = 7.7%. Refer to Table C.3.1.
Reliability of the Method [ILV]	ILV Study No. 00-0024 was conducted to verify the reliability of the method for the determination of ETU residues in meat. The values obtained are indicative that the method is reliable. Refer to Table C.3.1.	ILV Study No. 00-0026 was conducted to verify the reliability of the method for the determination of ETU residues in milk. The values obtained are indicative that the method is reliable. Refer to Table C.3.1.
Linearity	The method/detector response was linear (coefficient of determination, r^2= 0.999) within the range of 0.003-0.05 µg/mL for successful trials	

TABLE C.2.1. Characteristics for the Enforcement Analytical Methods Used for the Quantitation of ETU Residues in Milk and Beef Muscle.	
Specificity	The control chromatograms generally have no peaks above the chromatographic background, and the spiked sample chromatograms contain only the analyte peak of interest. Peaks were well defined and symmetrical. There appeared to be no carryover to the following chromatograms.

C.3. Independent Laboratory Validation

ILV studies were conducted for methods Meth-127 and Meth-128 by EN-CAS Analytical Laboratories (Winston-Salem, NC). Samples of untreated ground beef (30% fat content) and whole milk, obtained locally, were fortified with ETU at 0.001 and 0.01 ppm (LOQ and 10x LOQ) each. Five samples at each fortification level were analyzed according to the method procedures in Table B.2.1.

The methods were initially conducted as written with minor modifications including use of a different HPLC/ELCD system, minor adjustments to extract volumes, and use of plastic pH indicator strips rather than wide-range pH paper as recommended for testing. For Meth-127, samples of ground beef were initially reground with dry ice by the laboratory at some point before analysis, although the method write-up did not call for regrinding. The laboratory noted that for Meth-127 it was necessary to inject aliquots of matrix sample preparations in order to condition the HPLC system prior to injecting admissible standards and samples.

Meth-127: The first two trials were unsuccessful; some or all recoveries were <70% at both fortification levels. In contact with the method developers following the first trial, it was determined that the analysis time from extraction to HPLC injection may have been too lengthy and that the particle size of the ground beef may have been too small due to regrinding. In addition, the method developers recommended that all solids formed during concentration steps be transferred to the next step. In the second trial, a second analyst was added to reduce the sample preparation time, and samples of ground beef as received from the grocer were used. When unacceptable recoveries were obtained at the 0.01-ppm fortification level, a series of experiments was conducted which indicated that ETU losses were occurring primarily at the initial extraction step. As a result of these experiments, the following modifications were used for analysis of samples fortified at 0.001 ppm: the bottoms of the extraction jars were cooled on a bed of ice during extraction, and the pH adjustment step was modified to include dropwise addition of NH_4OH with swirling after each addition and the use of pH paper rather than plastic strips; the average recovery at 0.001 ppm was ~70.5%. The third trial, incorporating all of the modifications detailed above, was successful; recoveries are reported in Table C.3.1.

The following critical steps were identified by the ILV laboratory: (1) matrix priming of the HPLC system prior to sample injection to ensure acceptable ETU peak shape and consistent response; (2) use of the proper amount of ammonium hydroxide to reach and maintain pH 11-12 in the initial extraction solution and use of pH paper rather than plastic indicator strips; (3) external cooling during blending of the extraction mixture; and (4) transfer of any solids formed during the evaporative concentration steps to the next method step along with any transferred

liquid. The laboratory noted that all silanized glassware should be rinsed with acetone to prevent residual silanizing agent from suppressing instrument response.

The following changes to the method were recommended: (1) addition of a comment indicating that priming of the HPLC system with matrix injections may be necessary to achieve usable ETU peak shapes and responses; (2) addition of a recommendation that HPLC vials not be silanized (the method currently recommends that all glassware be silanized, but does not specifically address HPLC vials); (3) expansion of the description for the pH adjustment step to include the need to vigorously swirl the initial meat extract solution after addition of each portion of ammonium hydroxide is added to reach a stable pH, and a recommendation to use Hydrion wide-range pH paper to accurately measure pH; (4) addition of an alert to the reader to expect a significant quantity of solids to be deposited at each of the evaporative concentration steps and instruction to transfer these solids to the next method step along with the transferred liquid.

The ILV laboratory reported that the Sponsor Study Monitor was notified of the results of each sample set as they were completed, and pre-approved the experiments conducted during the second trial. The method developers were contacted as noted above following the failure of the first ILV trial. Documentation of communications was not included in the submission.

The ILV laboratory reported that a set of six meat samples can be prepared by one analyst in ~8-9 hours or by two analysts in ~6 hours, and an HPLC/ELCD run containing six standards and six samples can be completed overnight unattended.

Meth-128: The initial trial was unsuccessful. The ILV laboratory concluded that samples analyzed at the 0.001-ppm fortification level were not sufficiently centrifuged. The laboratory also reported that peak shape and analyte response varied substantially through the HPLC run, and attributed this to a continual increase in HPLC system priming caused by flushing the system with large volumes of mobile phase following injection of the sample matrix. Following unsuccessful analysis at the 0.001-ppm fortification level, conditions in the centrifugation step were monitored more closely for analysis at the 0.01-ppm fortification level, and it was discovered that substantial pre-run priming of the HPLC system with sample matrix injections was needed to obtain consistent peak shape and accurate results. The second trial, incorporating these modifications, was successful at both fortification levels; recoveries are reported in Table C.3.1.

Centrifugation of the initial extract and matrix priming of the HPLC system prior to sample injections were identified as critical steps. The laboratory also noted that the use of silanized HPLC vials appeared to affect responses for ETU calibration standards in preliminary testing, and concluded that residual silanizing reagent and/or byproducts remaining in the vials could reduce ETU response.

The ILV laboratory recommended the following changes to the method: (1) addition of a comment indicating that the supernatant following centrifugation of the milk extract should appear only slightly cloudy, and that if a substantial quantity of intractable milk solids is present after concentration of the extract, ETU recovery may be reduced; more rigorous centrifugation

conditions may be required; (2) addition of a comment indicating that priming of the HPLC system with matrix injections may be necessary to achieve acceptable ETU peak shapes and responses; and (3) addition of a recommendation that HPLC vials not be silanized (the method currently recommends that all glassware be silanized, but does not specifically address HPLC vials).

The ILV laboratory reported that the Sponsor Study Monitor was notified of the results of each sample set as they were completed, and pre-approved the experiments conducted during the second trial. Contact with the method developers following the failure of the first ILV trial at the first fortification level (0.01 ppm) yielded the following recommendations: (1) that the centrifugation conditions described in the method be followed as closely as possible; (2) that examination and adjustment of the ELCD might be needed to improve recoveries; and (3) that priming the HPLC system with matrix injections had been found to improve responses for ETU. Documentation of communications was not included in the submission.

The ILV laboratory reported that a set of six milk samples can be prepared by one analyst in one day (~8 hours), and an HPLC/ELCD run containing six standards and six samples can be run overnight unattended.

TABLE C.3.1. Recovery Results Obtained by an Independent Laboratory Validation of the Enforcement Methods for the Determination of ETU in Milk and Beef Muscle.

Matrix	Trial No.	Spiking Level (ppm)	Recoveries Obtained (%)	Mean Recovery ± Std. Dev. [CV] (%)
Meth-127: Ground beef	3	0.001	71.8, 73.2, 74.2, 76.3, 88.3	76.8 ± 6.7 [8.7]
		0.01	69.2, 69.5, 70.0, 73.9, 82.9	73.1 ± 5.8 [7.9]
Meth-128: Milk	2	0.001	73.1, 78.5, 81.1, 81.8, 83.7	79.6 ± 4.1 [5.2]
		0.01	82.3, 87.4, 87.7, 90.7, 96.1	88.8 ± 5.1[5.7]

D. CONCLUSION

Adequate ILV data have been submitted for HPLC/ELCD methods Meth-127 and Meth-128 reflecting analysis of ground beef and milk, respectively, both fortified at 0.001 ppm (LOQ) and 0.01 ppm (10x LOQ). The ILV laboratory recommended several changes to the methods. No method validation data or radiovalidation data were included in the submissions.

E. REFERENCES

DEB No.: 4368
Subject: Mancozeb – Unregistered Technical – Rohm & Haas Company – Product Chemistry Data Dated August 30, 1988.
From: G. Makhijani
To: L. Rossi and R. Engler
Date: 11/9/88
MRIDs: None

DEB No.:	4689
Subject:	Mancozeb – Unregistered Technical – Rohm & Haas Company – Product Chemistry Data Dated November 18, 1988.
From:	G. Makhijani
To:	L. Rossi and R. Engler
Date:	12/16/88
MRIDs:	40898301 and 40898302

DEB No.:	4750
Subject:	Mancozeb – Unregistered Technical and Manufacturing Use Products – E.I. DuPont de Nemours and Company, Inc. – Product Chemistry Data Dated December 2 1988.
From:	G. Makhijani
To:	L. Rossi and R. Engler
Date:	12/16/88
MRIDs:	None

DP#:	305815
Subject:	Mancozeb: Residue Chemistry Chapter of the Reregistration Eligibility Decision; Chemical No. 014504
From:	C. Olinger
To:	K. Farwell and T. Spears
Dated:	6/14/05
MRIDs:	None

F. DOCUMENT TRACKING

RDI: COlinger (7/6/09)
Petition Number: Not Applicable
DP#: 352531
PC Code: 014504

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